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Endotheliopathy of trauma is an on-scene phenomenon, and is associated with multiple organ dysfunction syndrome: a prospective observational study

Running head: Prehospital endotheliopathy of trauma

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ABSTRACT

Background

Trauma patients are vulnerable to coagulopathy and inflammatory dysfunction associated with endotheliopathy of trauma (EoT). *In vitro* evidence has suggested that tranexamic acid (TXA) may ameliorate endotheliopathy. We aimed to investigate how soon after injury EoT occurs, its association with multiple organ dysfunction syndrome (MODS), and whether TXA ameliorates it.

Methods

A prospective observational study included 91 trauma patients enrolled within 60 minutes of injury and 19 healthy controls. Blood was sampled on enrolment and again 4–12 hours later. ELISAs measured serum concentrations of syndecan-1 and thrombomodulin as biomarkers of EoT. MODS was compared between groups according to biomarker dynamics: (a) persistently abnormal; (b) abnormal to normal; and (c) persistently normal. Timing of EoT was estimated by plotting biomarker data against time, and then fitting generalized additive models. Biomarker dynamics were compared between those who did or did not received prehospital TXA.

Results

Median age was 38 (IQR 24–55) years; 78/91 were male. Median injury severity score (ISS) was 22 (IQR 12–36). EoT was estimated to occur at 5–8 minutes after injury. There were no significant differences in ISS between those with or without prehospital EoT. 42 patients developed MODS; 31/42 with persistently abnormal; 8/42 with abnormal to normal; and 3/42 with persistently normal biomarkers; $p<0.05$. There were no significant differences between TXA and non-TXA groups.

Conclusions

EoT was present at the scene of injury. MODS was more likely when biomarkers of EoT were persistently raised. There were no significant differences between TXA and no TXA groups. Prehospital interventions aimed at endothelial restoration may represent a clinically meaningful target for prehospital resuscitation.

Key words

Endothelium; syndecan-1; glycocalyx; thrombomodulin; trauma; endotheliopathy; prehospital

INTRODUCTION

Trauma induced coagulopathy (TIC) has been observed in injured patients on arrival in hospital(1), and within the prehospital environment(2, 3), suggesting that it may occur soon after injury. Abnormal inflammatory function following trauma has also been observed on admission to hospital(4), and we have recently reported that this occurs within the first hour following injury(5). Activation of the interconnected inflammatory and coagulation pathways are influenced by a "genomic storm" that occurs following injury, mimicking the response to critical inflammatory stress(6). This may include the disruption of the endothelial barrier, which has been proposed as a unifying mechanism for these processes following trauma(7). There have been no investigations of this specific process in the prehospital setting. Data regarding the timing and nature of endotheliopathy following injury and before arrival in hospital may facilitate a greater understanding of the mechanisms of coagulopathy and inflammatory disorder following trauma.

The endothelial layer consists of endothelial cells, their basal lamina and the endothelial glycocalyx that lines the luminal surface. Endothelial injury and glycocalyx shedding may occur following trauma as a consequence of its exposure to circulating damage-associated molecular patterns (DAMPs)(8), neutrophil extracellular traps(9), sympathoadrenal activation(10), hypovolaemia(11), and ischemia(12). In injured patients, soluble thrombomodulin and syndecan-1 have been used as surrogate markers of endothelial cell injury and glycocalyx shedding respectively(10, 13). These biomarkers have been observed in abnormal concentrations in blood samples acquired from patients at hospital admission(13), but it is unknown exactly when endotheliopathy occurs following a traumatic insult. If endothelial activation and microcirculatory dysfunction are to be confirmed as a

unifying mechanism for the pathological inflammatory processes following trauma(7), then it would be expected that endotheliopathy precedes, or concurrently occurs, with coagulopathic and inflammatory changes following injury. We sought to investigate endotheliopathy of trauma (EoT) within a cohort of trauma patients that we recently observed to have early inflammatory and immune cell dysregulation(5).

A recent *in vitro* study of the EoT demonstrated that when injured human umbilical vein endothelial cells were exposed to tranexamic acid (TXA), concentrations of both thrombomodulin and syndecan-1 (used as markers of endotheliopathy) were reduced significantly more than for those cells without TXA(14). This study generated the hypothesis that TXA may play some role in the amelioration of the EoT, but this has not been tested in the clinical setting.

The current study measured biomarkers of endothelial cell injury (thrombomodulin) and glycocalyx shedding (syndecan-1) as surrogate markers of EoT. The aim of the study was to provide an estimation of the time of onset of EoT post-injury, and to investigate whether the evolution of EoT between the scene of injury and hospital admission is associated with subsequent organ failure. Furthermore, since these two time points represent "before" and "after" treatment with TXA, we tested the hypothesis that TXA might reduce biomarkers of EoT as has been reported *in vitro*(14).

We hypothesised that there would be an early rise in biomarkers of EoT following trauma, which would be associated with poorer outcomes, and that patients who received TXA would have a greater decrease in biomarker levels than those who did not receive TXA.

METHODS

Study design and setting

A prospective longitudinal observational study was undertaken using a prehospital design described previously(5); trauma patients were enrolled during prehospital evacuation, as soon after injury as possible by paramedic personnel across a large UK major trauma network. After being conveyed to the regional Major (Level 1) Trauma Centre (University Hospitals Birmingham NHS Foundation Trust), study participants were followed up by embedded trauma research personnel. The current study includes patients from the Brain Biomarkers After Trauma Study (BBATS); Research Ethics Committee (REC) approval was granted prior to the start of the study (REC 5, Wales, Ref. 13/WA/0399). This study is reported according to the STROBE guidance for observational studies. Patients were enrolled from May 2014 to February 2017.

Prehospital blood products were not available in this trauma network during the study period. Prehospital TXA delivery was given according to a specific protocol that included injured patients with any of the following: systolic blood pressure <90mmHg; heart rate >100bpm; risk of significant haemorrhage; or who required intravenous fluid therapy. Patients were not given TXA if more than 3 hours had passed since injury, if they had an isolated head injury, a known history of convulsions, or hypersensitivity to TXA.

Study participants

Trauma patients with the likelihood of an Injury Severity Score (ISS) of 8 or higher were eligible for inclusion, regardless of type of injury. Since ISS is not usually calculated until after the patient has left hospital, specific training was delivered to the prehospital practitioners within the study region before the start of the study, so that prediction of likely

ISS could be made as appropriate. Patients were excluded if they were pregnant, prisoners, or under the age of 16. Patients could only be enrolled if there was a peripheral venous sample of blood taken within 60 minutes from time of injury. All eligible patients were screened by dedicated research staff on arrival in ED to minimise the risk of selection bias. For the purposes of the current study, patients with isolated traumatic brain injury were excluded.

Capacity and consent

Due to the injuries sustained and requirement for swift emergency evacuation, it was considered that patients would not have capacity to consent. The REC-approved protocol was undertaken according to the World Medical Association Declaration of Helsinki, and the Mental Capacity Act 2005. In brief, a close relative or friend (Personal Consultee) was asked to assent for the patient's participation in the study, based on their prior knowledge of the participant's wishes. Once the patient regained capacity, they were approached in order to gain consent for the retention of data already obtained, and for continued follow up. If a patient did not regain capacity, the permission from their Personal Consultee to participate in the study remained extant.

Blood sampling and storage

Peripheral blood samples were taken during the prehospital evacuation of all patients within 60 minutes of injury. The timing of this sample was meticulously recorded in contemporaneous records by prehospital personnel (designated as the "prehospital" time point). A further peripheral blood sample was taken between 4 and 12 hours after injury (designated the "in-hospital" time point). Blood samples were collected into BD Vacutainers® (Becton Dickinson, Oxford, UK) containing z-serum clotting activator. Following a 30-minute incubation at room temperature, samples were centrifuged at 4^{o}C for

10 minutes at 1,620 x g. Aliquots were stored at -80^{o}C until analysed. Serum samples were acquired and stored for 19 "healthy control" (HC) volunteers, who were matched as a group for sex and age to the study cohort. All HCs had declared themselves to be in good health, and not currently under any medical investigations or treatment, and had no chronic diseases.

Enzyme-linked immunosorbent assays

Commercially available ELISAs were used to detect concentrations of syndecan-1 (CD 138) (Abcam, ab46506, Cambridge, MA) and thrombomodulin (CD 141) (Abcam, ab46508, Cambridge, MA) in the serum samples. Analysis was undertaken in accordance with the relevant protocols, including the use of control and blank wells in order to validate the results.

Data collection

Data was recorded prospectively for all patients, and then confirmed using electronic and paper records. Data included patient demographics (age, gender), and mechanism of injury. Injury severity scores (ISS) were acquired from the centralised UK Trauma Audit and Research Network after discharge from hospital. Physiological parameters in ED included lowest systolic blood pressure and associated heart rate, and Glasgow Coma Scale. Serum lactate and base deficit in ED were also recorded. Timings of blood samples were collected contemporaneously by prehospital practitioners. Delivery of TXA between the first and second time points was recorded in order to dichotomise patients into those who had received TXA and those who had not.

Outcomes

The primary outcome for this study was development of multiple organ dysfunction syndrome (MODS), defined as a sequential organ failure assessment (SOFA) score of 6 or higher on 2 or more consecutive days during their admission in hospital after the first 48h following injury(4, 5). Mortality within 90 days was also recorded for all patients.

Data analysis

A Shapiro-Wilk test was used to determine the normality of continuous data. Non-normal data are presented as median and interquartile range (IQR). Syndecan-1 and thrombomodulin concentrations were considered abnormal if they were above the 97.5^{th} percentile of HC levels; patients were considered to have endotheliopathy if they had abnormal values for either biomarker (syndecan-1, thrombomodulin, or both). Correlation between non-normal continuous variables was undertaken using Spearman's rank correlation coefficient. Non-normal continuous data were compared between groups using a Kruskal-Wallis test, followed by pairwise comparisons between groups using Dunn's multiple comparisons test. Categorical data were compared using Fisher's exact test for pairwise comparisons, and chi-squared analysis for trends between groups, and reported as n/denominator. A *p*-value of <0.05 was considered significant. Where appropriate, further designation of significance was made by indicating when *p*-values were <0.01, <0.001 and <0.0001.

a. Estimation of the time of onset of endotheliopathy

It is highly unlikely that any study of humans will be able to determine the concentrations of biomarkers at the point of wounding, and therefore statistical modelling was considered the best opportunity to estimate the timing of biomarkers following injury. In order to determine the most likely time at which endothelial injury and glycocalyx shedding occur following

injury, the concentrations of thrombomodulin and syndecan-1 for all patients were plotted against time from injury. Syndecan-1 and thrombomodulin concentrations derived from HCs were plotted at the origin, on the assumption that trauma patients would have been within the same range prior to injury. Analysis was then performed by fitting generalized additive models to the data using penalized regression splines with smoothing parameters selected by residual maximum likelihood. This modelling approach accounts for the distinctly non-linear pattern of the data that cannot be adequately modelled with standard parametric regression approaches. For each biomarker, two curves were plotted: the group of "abnormal" values (higher than the 97.5th percentile of HCs) and the group of "normal" values which were between the 2.5th and 97.5th percentiles of HCs. The point at which the abnormal curve crossed the line of significance was considered to be an estimation for the point at which endotheliopathy occurred following injury.

b. Measuring the effects of TXA

Patients who had prehospital endotheliopathy were dichotomised into those who received TXA within the prehospital period (between time points) and those that did not. Biomarkers were then compared before and after treatment in order to determine whether there were any differences between the two groups.

c. Investigating the association between endotheliopathy and MODS

Patients with both pre- and in-hospital serum samples were categorised according to their biomarkers over the two time points: (a) those with persistent abnormal biomarkers; (b) those that had abnormal biomarkers that reduced to normal range; and (c) those with normal biomarkers throughout. The numbers of patients who developed MODS were compared

between these three groups. Furthermore, individual biomarkers were compared between those who developed MODS and those who did not.

RESULTS

Study participation

A total of 816 patients were screened for eligibility during the study period, of which 107 were potentially eligible patients who had blood samples taken within 60 minutes of injury. After 16 were excluded due to isolated traumatic brain injury, there were 91 patients included. The median time of blood sampling for the prehospital time point was 45 (IQR 33–55) minutes after injury. The median time of blood sampling for the second (in-hospital) time point was 5.5h (IQR 4.2h – 10.5h).

Enrolment and participation at each stage are illustrated in a flow diagram (Figure 1), with asterisks to indicate which denominator is used for each stage of data analysis. The prehospital blood samples were insufficient for analysis for 3 patients, and the in-hospital blood samples were insufficient for a further 9 patients, so that 79/91 patients had both pre- and in-hospital samples suitable for analysis. The denominators used for the different analyses differ accordingly:

(a) Analysis of the timing of EoT after injury: N=88 (all prehospital samples)
(b) Comparison of biomarkers according to outcomes (MODS and mortality); and comparison between TXA and non-TXA: N=91 (all patients).
(c) Comparison of patient groups according to dynamic changes in endotheliopathy between pre- and in-hospital time points: N=79 (all patients with both pre- and in-hospital samples)

Patient characteristics

The median age of all patients was 38 (IQR 24–55) years, and 78/91 were male. Patient characteristics are illustrated in Table 1, with comparison between patients with prehospital EoT and those without. There was a higher proportion of patients with blunt injury in the prehospital endotheliopathy group than those without prehospital endotheliopathy (60/71 *versus* 9/17; $p<0.01$). All other characteristics were not significantly different between the groups.

Table 1. Study participant characteristics according to presence of endotheliopathy at the prehospital time point

Patient characteristic	All (N=88)	Prehospital Endotheliopathy (N=71)	No prehospital endotheliopathy (N=17)	*p*-value
Age, years	33 (24–53)	38 (24–56)	25 (23 – 44)	0.343
Sex, male	75 (85)	61 (86)	14 (82)	0.993
Mechanism of injury				
Blunt	69 (78)	60 (85)	9 (53)	0.008*
Penetrating	19 (22)	11 (15)	8 (47)	
Injury Severity Score	23 (12–36)	24 (14–36)	16 (9–29)	0.267
Physiological parameters in ED				
Lowest SBP, mmHg	115 (99–124)	114 (99–124)	119 (102–124)	0.666
Heart rate, min^{-1}	88 (76–99)	87 (75–99)	89 (78–103)	0.629
Glasgow Coma Scale	13 (5–15)	13 (4–15)	15 (10–15)	0.150
Serum lactate, mmol/l	4.1 (2.5–6.5)	4.2 (2.7–6.7)	3.5 (2.0–5.5)	0.259
Base deficit, mmol/l	-3 (-6 – -1)	-3 (-6 – -1)	-3 (-6 – -1)	0.438

Summary data are presented as median (interquartile range); categorical data are reported as N (%)

ED: Emergency Department; SBP: systolic blood pressure

*Significant according to Fisher's exact test

Presence of endotheliopathy

According to serum concentrations of thrombomodulin and syndecan-1 for HCs and patients, there was a significant difference between them at both time points, for both biomarkers, as illustrated in Table 2. According to the presence of abnormal syndecan-1 or thrombomodulin concentrations, prehospital and in-hospital endotheliopathy were present in 71/88 (81%) and 51/82 (62%) patients respectively. For patients with samples available at both time points (N=79) (Figure 1), 50/79 had persistently abnormal biomarkers, 18/79 had abnormal biomarkers that returned to normal, and 11/79 had normal biomarkers throughout; their characteristics are illustrated in Table 3. There was a significant difference across groups for serum lactate, base deficit, and SBP (all $p<0.05$). Of all patients with biomarkers at both time points, there were 20/79 (25%) who received blood products between the time points. There was a significant difference in transfusion requirement between these groups according to biomarker dynamics; almost all of those requiring transfusion were in the group that had persistently abnormal biomarkers ($p < 0.01$) (Table 3).

Table 2. Serum syndecan-1 and thrombomodulin concentrations for healthy controls and patients according to time point.

Biomarker, ng/ml	Healthy controls (N=19)	Prehospital (N=88)	In-hospital (N=82)
Syndecan-1	30 (20–44)	59 (39 – 140)***	53 (28 – 150)*
Thrombomodulin	2.9 (2.2 – 3.4)	4.9 (3.8 – 6.4)***	4 (3.4 – 5.1)**

Data are presented as median (interquartile range), ng/ml

*$p<0.01$, **$p<0.001$, and ***$p<0.0001$ when compared to healthy controls using Dunn's multiple comparisons test

Table 3. Patient characteristics according to change in serum syndecan-1 and thrombomodulin concentrations between the prehospital and in-hospital time points

Patient characteristic	Abnormal-abnormal (N=50)	Abnormal-normal (N=18)	Normal-normal (N=11)	*p*-value
Age, years	39 (25–63)	36 (24–45)	25 (23–48)	0.417
Sex, male	42 (84)	16 (89)	9 (82)	0.974
Mechanism of injury				
Blunt	39 (78)	17 (94)	7 (64)	0.729
Penetrating	11 (22)	1 (6)	4 (36)	
Injury Severity Score	25 (15–38)	29 (16–38)	17 (9–29)	0.230
Physiological parameters in ED				
Lowest SBP, mmHg	113 (95–122)	119 (105–129)	122 (106–134)	0.030*
Heart rate, min^{-1}	87 (80–99)	93 (81–104)	88 (76–104)	0.627
Glasgow Coma Scale	13 (3–14)	12 (6–14)	15 (11–15)	0.083
Serum lactate, mmol/l	4.8 (3.0–7.2)	3.7 (2.5–6.4)	2.4 (1.3–4.6)	0.037*
Base deficit, mmol/l	-4 (-8 – -2)	-3 (-6 – -1)	-2 (-3 – 0)	0.023*
Blood product requirement				
All patients transfused	19 (38)	0 (0)	1 (10)	0.003**
Patients given RBCs	19 (38)	0 (0)	1 (10)	0.003**
Patients given FFP	12 (24)	0 (0)	0 (0)	0.017**

Summary data are presented as median (interquartile range); categorical data are reported as N (%)

ED: Emergency Department; SBP: systolic blood pressure; RBC: red blood cells; FFP: fresh frozen plasma

*Significant according to Kruskal-Wallis test

**Significant according to Chi-squared test

Endotheliopathy occurs within minutes of injury

There was evidence of glycocalyx shedding and endothelial cell damage within the prehospital (<60 min) phase amongst this cohort of patients. Before statistical modelling, the first recorded abnormal syndecan-1 and thrombomodulin concentrations amongst the patient cohort were observed at 17 and 18 minutes following injury respectively (Figure 2). When

the statistical model was used, glycocalyx shedding and endothelial cell injury were estimated to occur at approximately 5 and 8 minutes following injury respectively (Figure 2).

Endotheliopathy is associated with poor perfusion

When prehospital and in-hospital concentrations of syndecan-1 and thrombomodulin were compared to the serum lactate during ED resuscitation (as a surrogate marker of microcirculatory perfusion), there was a significant correlation with both prehospital and in-hospital syndecan-1 (Figures 3a and 3b respectively), and in-hospital thrombomodulin (Figure 3d); all $p<0.05$. However, there was no significant correlation between lactate and prehospital thrombomodulin concentration (Figure 3c).

Endotheliopathy is associated with MODS

Figure 4 illustrates the biomarker levels of patients according to whether they developed MODS. When patients who developed MODS were compared to those without MODS, the former had significantly higher syndecan-1 concentrations at the prehospital (91 (IQR 48–184) ng/ml *vs* 47 (IQR 33–109) ng/ml; $p<0.05$) and in-hospital (64 (IQR 42–199) ng/ml *vs* 38 (IQR 25–98) ng/ml; $p<0.01$) time points. For patients with MODS, the pre- and in-hospital biomarker levels were both significantly higher than those of HCs (30 (IQR 19–44) ng/ml); $p<0.0001$ and $p<0.001$ respectively. Patients without MODS also had significantly higher syndecan-1 concentrations in the prehospital samples ($p<0.05$) but not in the in-hospital samples ($p=0.216$) (Figure 4a).

Patients with MODS had significantly higher thrombomodulin concentrations at the prehospital (5.4 (IQR 4.0–7.1) ng/ml *vs* 4.5 (IQR 3.4–5.9) ng/ml; $p<0.05$) and in-hospital (4.5 (IQR 3.6–5.5) ng/ml *vs* 3.6 (IQR 2.9–4.5) ng/ml; $p<0.01$) time points. For patients with MODS, the pre- and in-hospital biomarker levels were both significantly higher than those of

HCs (2.9 (IQR 2.2–3.5) ng/ml); $p<0.0001$ for both. Patients without MODS had significantly higher thrombomodulin concentrations in the prehospital samples ($p<0.0001$) but not in the in-hospital samples (p=0.065) (Figure 4b)

Changes in biomarkers are associated with MODS

For those patients with both prehospital and in-hospital samples (n=79), 42/79 patients developed MODS. These included 31/42 who had persistently abnormal biomarkers, 8/42 had abnormal prehospital biomarkers that normalised, and 3/42 had normal biomarkers at both time points; $p<0.05$.

When patients were compared according to dynamic change in biomarkers, MODS developed in 31/50 in the group that had persistently abnormal biomarkers, 8/18 in the group that had abnormal biomarkers that returned to normal range, and 3/11 in the group that had normal biomarkers throughout; $p < 0.05$ (Figure 5a). When syndecan-1 and thrombomodulin were examined separately, there was a similarly significant trend for MODS according to both biomarkers; $p<0.05$ and $p<0.01$ respectively (Supplementary Figure 1).

There were 12 patients who died. There was no significant difference in mortality within groups according to dynamic changes in biomarkers; 9/50 with persistently abnormal biomarkers, 2/18 of those with abnormal biomarkers that returned to normal range, and 1/11 of those with normal biomarkers throughout died; $p = 0.652$ (Figure 5b).

TXA did not influence biomarkers of endotheliopathy

Of all patients, there were 55/91 who received prehospital TXA, 35/91 did not, and one patient was randomized to either TXA or placebo as part of the CRASH-3 study(15). There were 38 patients with abnormal prehospital syndecan-1, of which 23/38 received TXA. There were no differences in syndecan-1 concentration at either time point according to TXA group

(Figure 6a). There were 43 patients with abnormal prehospital thrombomodulin, of which 25/43 received TXA. There were similarly no differences in thrombomodulin concentrations at either time point according to TXA group (Figure 6b).

DISCUSSION

The main finding of this study is that trauma-induced endotheliopathy occurred within minutes of injury, and failure of abnormal biomarkers to return to normal was associated with a higher burden of organ dysfunction. MODS was associated with higher syndecan-1 and thrombomodulin at both prehospital and in-hospital time points, but there was no association between delivery of TXA and biomarker levels. Endotheliopathy is likely to occur rapidly following injury, with observed biomarker levels raised at 17 minutes within the patient cohort, and statistical modeling of data predicting that this occurs within 5 – 8 minutes of injury. These data are in keeping with the hypothesis that endotheliopathy is a common mechanism(7, 16), and may partly explain why phenomena such as TIC and inflammation have been recorded in the prehospital environment and on arrival of patients in hospital. These data are also in keeping with previous evidence that high syndecan-1 is associated with poorer outcomes amongst trauma patients, even when taken very early after patient admission to hospital(17).

Our observations of early endotheliopathy following injury are in keeping with the current understanding of the mechanisms of early coagulopathy, since glycocalyx shedding and endothelial cell injury stimulate the generation of thrombin and activation of protein-C, with subsequent hyperfibrinolysis(18). Since inflammatory derangement following cellular injury may also be associated with subsequent sepsis(19) and organ failure(4), early reversal of these interconnected processes has the potential to improve outcomes. The patients that

developed MODS in this study came from the same cohort that showed inflammatory and immune cell dysregulation in our previous detailed study of immune function, with raised levels of IL-6, IL-8, and TNFa at both prehospital and in-hospital time points when compared to healthy controls(5). Together, these data support the hypothesis that there is an early and important relationship between endothelial injury and altered immune function following trauma, and that these derangements occur within minutes of injury. Multiple studies have reported independent associations between high injury severity, high plasma adrenalin level, and high circulating syndecan-1 and thrombomodulin levels, suggesting that sympatho-adrenal hyper-activation following shock may be a key driving factor in this process(5).

During the resuscitation of critically unwell trauma patients, lactate has been shown to be a useful biomarker of perfusion as a one-off reading and also in terms of progression between values (as lactate clearance),(20). In a similar manner, we present one-off individual biomarkers of endotheliopathy, but also progression of biomarkers during resuscitation, where the normalization of biomarkers are taken as a surrogate marker of endothelial restoration. In the current study, MODS was associated with non-reversal of endotheliopathy, and less common amongst those with endothelial biomarkers that returned to normal range. Restoration of the injured endothelium may therefore represent a meaningful clinical target for goal directed therapy during prehospital evacuation. Deliberate targeting of endothelial integrity is not currently undertaken in clinical practice. Plasma-based fluid resuscitation represents one possible treatment of choice for patients in this context, since both preclinical(21) and clinical(22) investigations have reported restoration of the endothelial glycocalyx after its delivery. The current study was non-randomized, and patients who had received plasma were more likely to be in the group that had persistently abnormal biomarkers. Although there were no significant differences in ISS between groups, a greater

requirement for blood product transfusion in this group suggests a higher number of patients with hemorrhagic trauma. A similar selection bias has been reported in observational studies of prehospital blood products, limiting meaningful clinical interpretation(23). These data cannot therefore be used to determine the efficacy of plasma-based resuscitation in the mitigation of endotheliopathy of trauma; further clinical investigations are required to test the hypothesis that plasma may allow endothelial restoration, especially if randomized controlled trials of prehospital plasma-based fluid resuscitation report improved clinical outcomes.

The need for prehospital research has been emphasised by the World Health Organisation(24) and the Institute of Medicine of the National Academies(25), and may enable questions to be addressed that are not necessarily answered by preclinical studies(26). Although the old concept of the "golden hour" may not have as much of a bearing on outcomes as it was first supposed(27), it appears to represent a time during which a cascade of important pathological processes occur following injury, very close to the point of wounding. The data from this study have been obtained within the prehospital period, within the first hour following injury. Rather than waiting until arrival in hospital to attempt to address pathological processes, there may be some justification for working towards earlier, and more bespoke, intervention for critically unwell patients(28). Such an approach has already been advocated in terms of remote damage control resuscitation(29), and the consideration of the endothelium and blood together as an "organ" during resuscitation following trauma(30).

The derangement of fibrinolysis has been considered as a target for trauma resuscitation in bleeding patients, such as with tranexamic acid(31) and fibrinogen concentrate(32), but there are no clinical therapies directly targeting the restoration of the glycocalyx or endothelial integrity. We were unable to confirm recent *in vitro* findings that early delivery of TXA can

reduce the burden of endotheliopathy following injury(14, 33). This may be due to the observational study design, and the heterogeneity in patient injury patterns and severity, as shown by the large confidence intervals in the data. Further investigation may be warranted on a larger scale, with a more homogenous population, using randomization to reduce the risk of selection bias.

More than half of patients who did not reverse their endotheliopathy went on to develop multiple organ failure. Although causality cannot be demonstrated in the current study, the relationship is in keeping with previous descriptions of early inflammatory dysfunction and MODS(4, 5). The reason that some patients have biomarkers that are restored to normal range but others do not (or indeed why some have normal biomarkers throughout) is unknown, and was not related to ISS within this study cohort. There was evidence that serum lactate, base deficit and SBP were significantly different between these groups which may imply that the degree of shock and perfusion are related to endothelial integrity – a finding supported by the relationship between biomarkers and serum lactate in our study. It is notable that although this association was statistically significant, the correlation was not perfect, suggesting that there is a complex relationship between perfusion and endothelial integrity, with other confounding factors in a heterogeneous trauma population. Further mechanistic studies are required to investigate the precise relationships between the endothelium, perfusion, and subsequent organ function following trauma.

It is likely that there are some genetic factors in the ability of the endothelium to respond to trauma and resuscitation, and early biomarkers may be one way of detecting these predispositions. Other biomarkers in trauma have been proposed as prognostic indicators(34), but these are not commonly used for trauma triage or goal directed therapy in clinical

practice. The utilization of such biomarkers—including those that may indicate endotheliopathy—remains a tantalizing potential avenue for future trials. If strategies to treat the endothelium are planned in the future, it seems that early treatment (within the prehospital environment) would be warranted since the current study suggests that early reversal (within 12 hours) is associated with lower morbidity.

Limitations

The current study is limited by its number of patients when analyzing differences in relatively rare occurrences such as mortality; although there appears to be a non-survival trend in the same direction as MODS, this was not found to be statistically significant. Not all patients had samples available from both time points. These patients were excluded during subgroup analysis, which gives potential risk of selection bias. The style of opportunistic study enrolment during prehospital evacuation makes the study at risk of selection bias, since only a proportion of eligible patients were recruited into the study. This risk was reduced by the maintenance of a screening log to ensure that "missed" patients were not significantly different in demographics and injury patterns than included patients.

The statistical modeling performed to evaluate the timing of endotheliopathy should be considered as an estimation only, since it requires several assumptions that have the potential to be biased; it was assumed that patients had similar biomarkers to HCs prior to injury, and that biomarker levels above the 97.5^{th} percentile of HCs represented the development of endotheliopathy. These assumptions were required for a pragmatic estimation, since time-of-injury sampling is unlikely to be ethically justifiable or logistically realized.

The reliability of out-of-hospital blood samples has been questioned in simulations of "prehospital" delay(35), which has the potential to bias results based on time difference

between sampling and arrival hospital. Since this was a pragmatic, real-life study, such a risk may exist, but was kept to a minimum by maintaining strict and consistent preparation techniques as soon as the sample was received.

We report that endothelial cell injury and glycocalyx shedding were observed within minutes following injury, and that statistical modeling predicted that this may occur at approximately 5–8 minutes. Failure to reverse prehospital endotheliopathy is associated with poorer clinical outcome in terms of multiple organ dysfunction syndrome. Biomarker dynamics did not appear to be related to injury severity. In our non-randomized study, TXA was not associated with reduced levels of biomarkers of endotheliopathy. These findings, together with other reports of early coagulopathy and inflammatory dysregulation, suggest that early interventions aimed at restoration of the endothelium may represent a clinically meaningful target for resuscitation. The most appropriate treatment for this purpose in the clinical context remains uncertain.

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Figure 1. Flow diagram of patient enrolment and participation at each stage of analysis. The denominators for analysis are: *N=91 for analysis of biomarkers according to multiple organ dysfunction syndrome (MODS) and mortality, and comparison of tranexamic acid (TXA) and non-TXA; **N=88 for analysis of timing of endotheliopathy; and ***N=79 for analysis of dynamic changes in endotheliopathy between time points

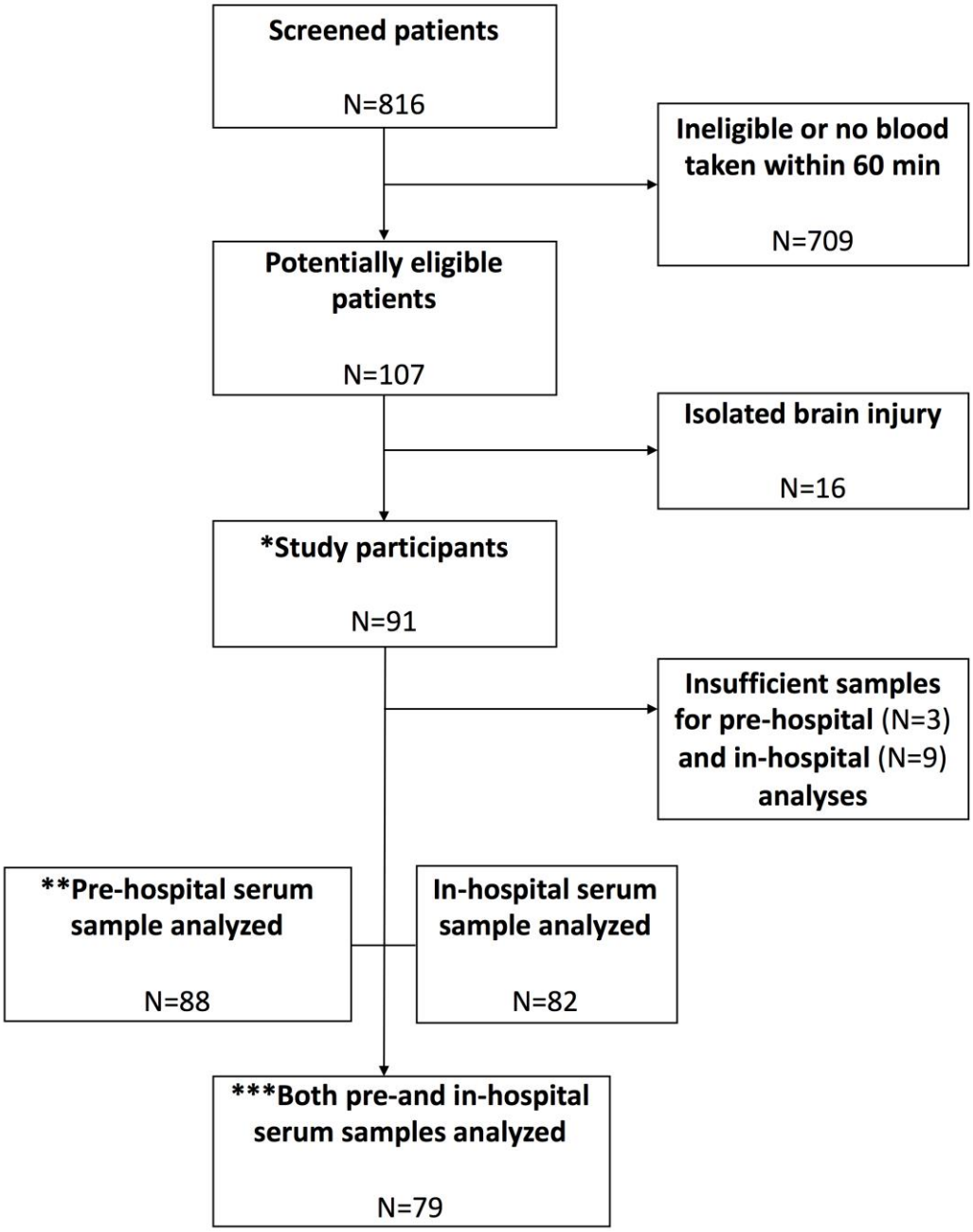


Figure 2. Concentrations of (a) syndecan-1 and (b) thrombomodulin in relation to time of injury.

Healthy controls are plotted at time = 0 min. Patients are divided into those with elevated concentrations of the biomarker (>97.5th percentile of healthy controls), and those with normal concentrations (2.5th – 97.5th percentiles of healthy controls). For each curve the central trend line represents the average values, with the upper and lower trend lines representing the 2.5th and 97.5th percentiles of observed values. The vertical interrupted red line indicates the time at which biomarkers are likely to be first elevated.

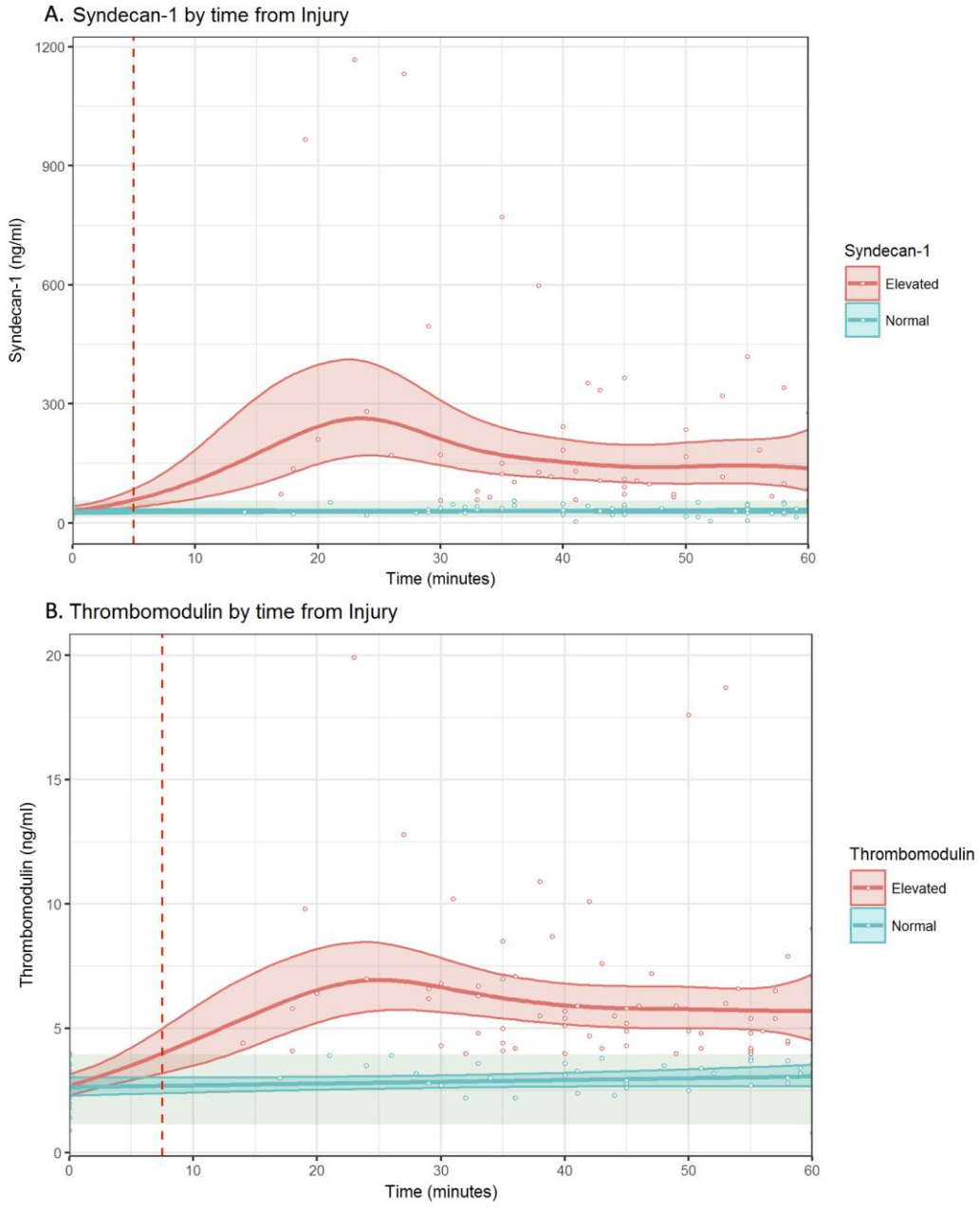


Figure 3. Concentrations of Syndecan-1 and thrombomodulin in relation to lactate concentration during ED resuscitation; including (a) prehospital syndecan-1; (b) in-hospital syndecan-1; (c) prehospital thrombomodulin; and (d) in-hospital thrombomodulin.

Blue dashed lines represent 95% confidence intervals.

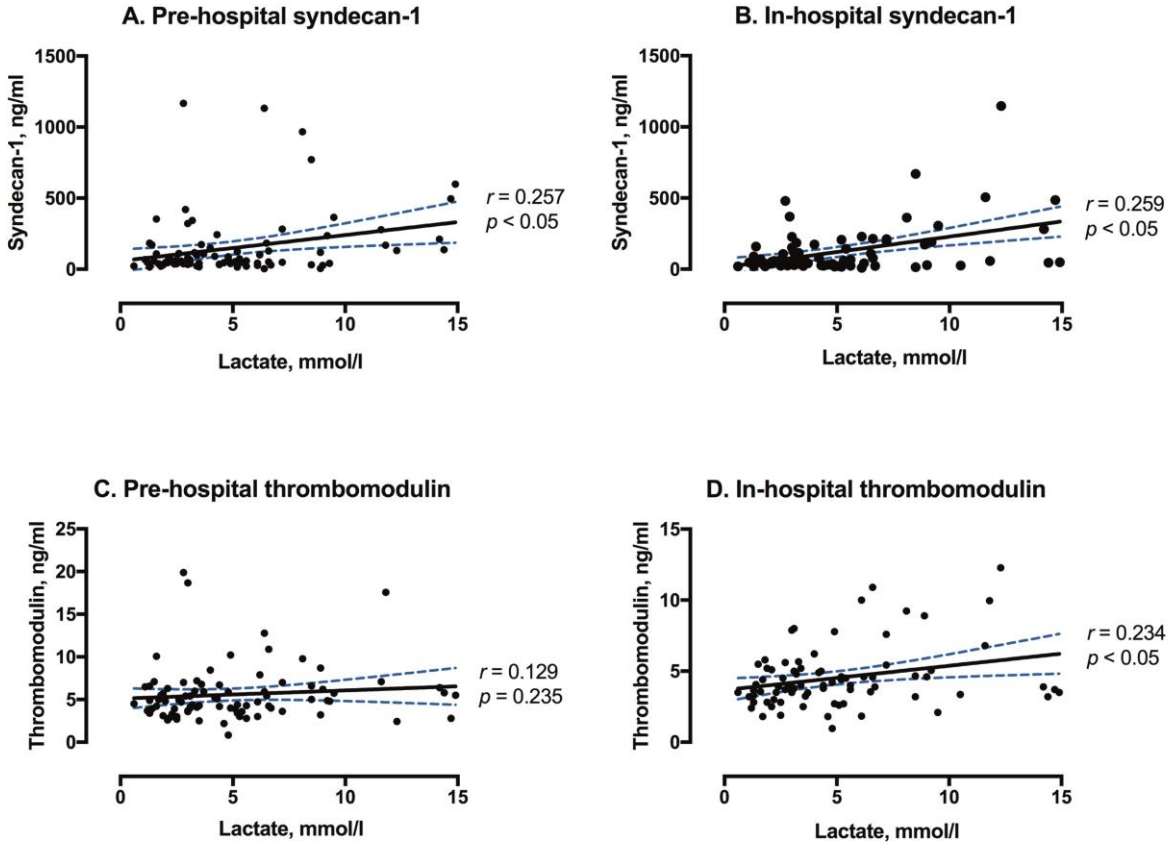


Figure 4. Concentrations of (a) syndecan-1 and (b) thrombomodulin according to time point and development of multiple organ dysfunction syndrome (MODS).

Healthy controls (HC) are displayed for comparison.

*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 *vs* HCs

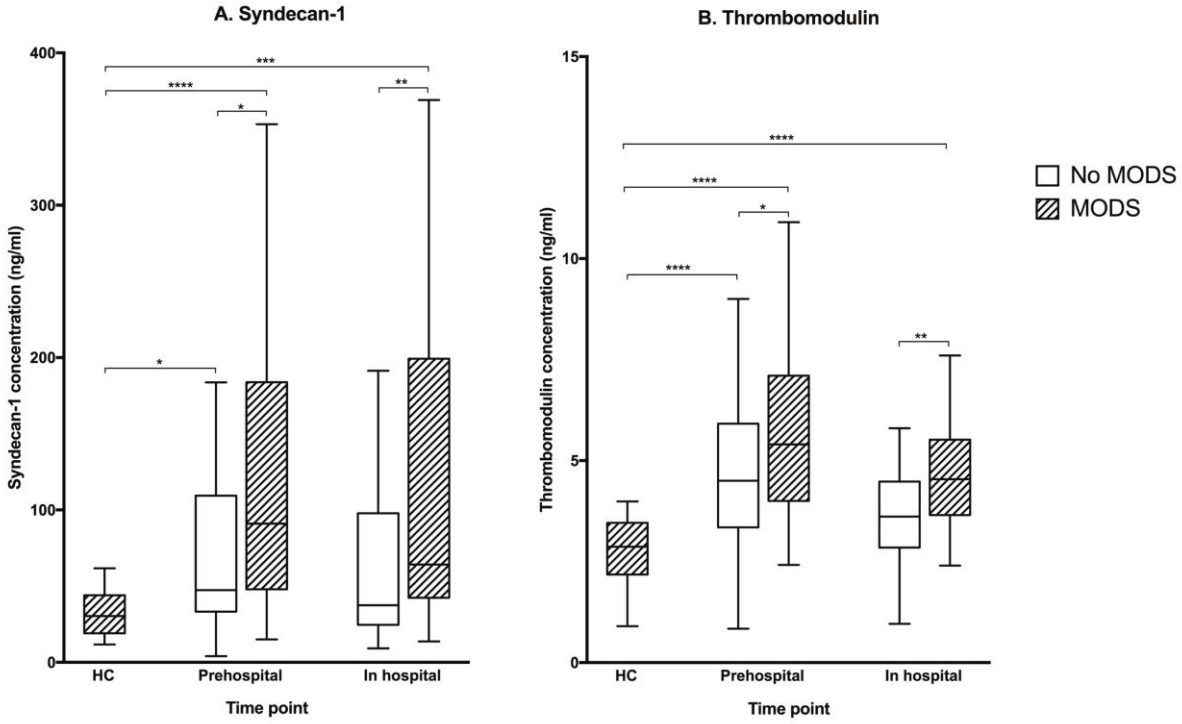
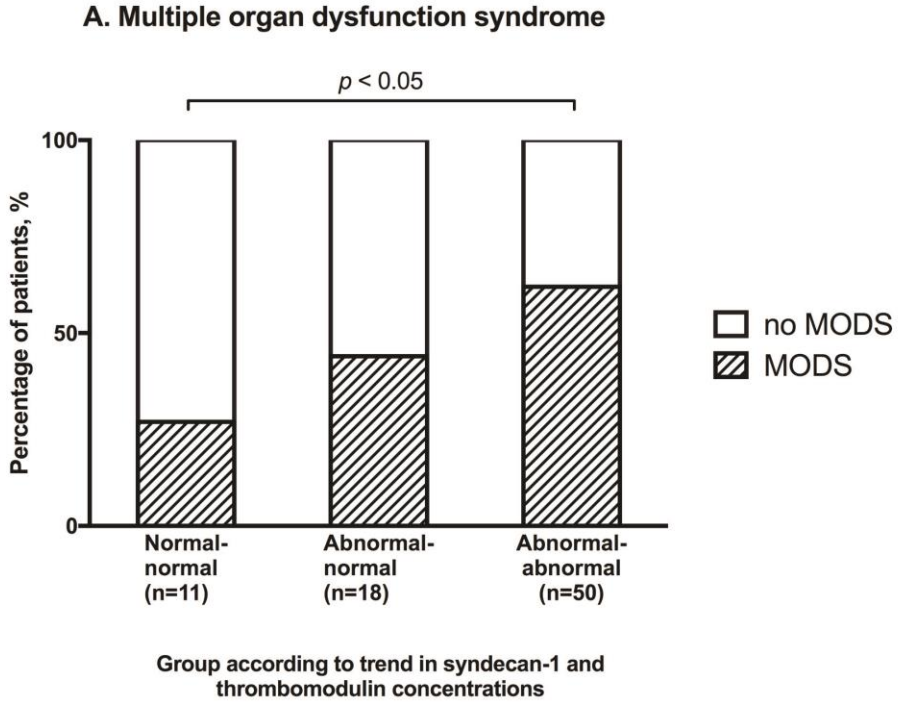


Figure 5. Presence of (a) multiple organ dysfunction syndrome (MODS) and (b) 90-day mortality amongst patients according to dynamic changes in levels of both syndecan-1 and thrombomodulin between prehospital and in-hospital time points.

Significance is indicated according to χ^2 test for trend.



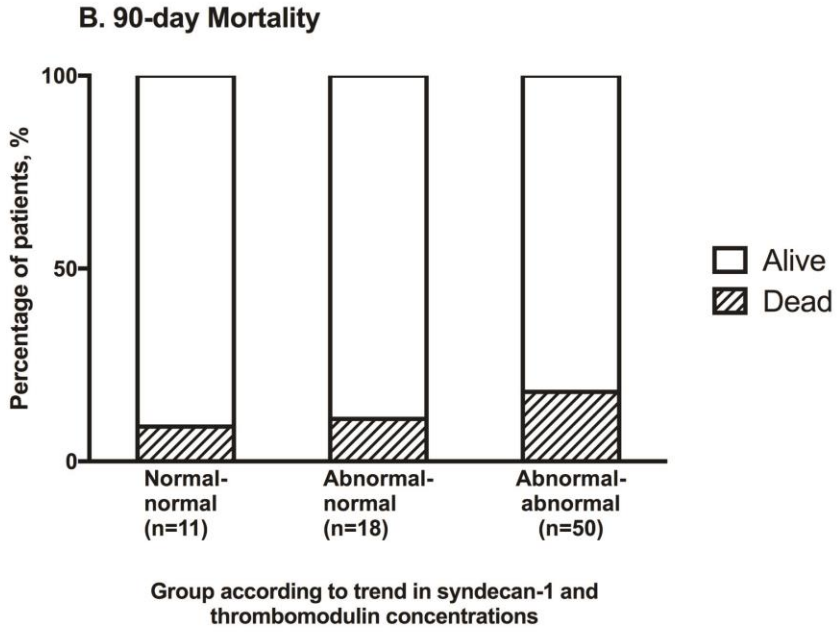
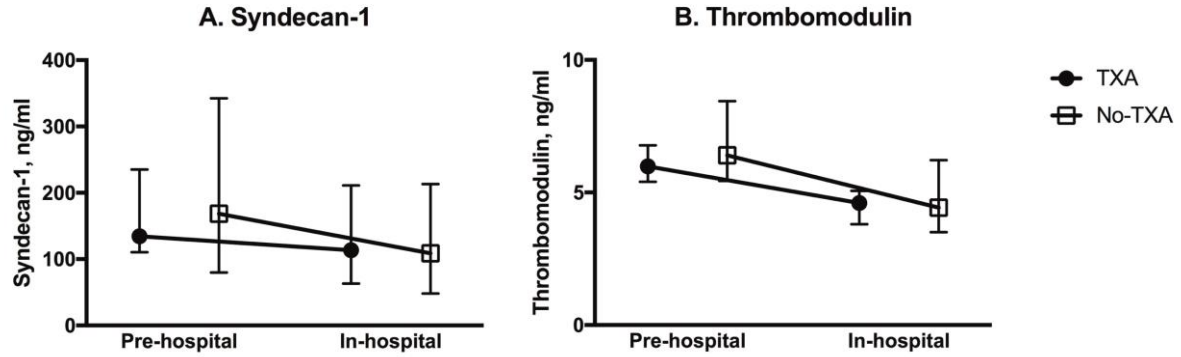


Figure 6. Prehospital and in-hospital levels of syndecan-1 and thrombomodulin according to whether TXA was given to the patient during the prehospital period.
Vertical bars represent 95% confidence intervals around the median.



Supplementary Figure 1. Presence of multiple organ dysfunction syndrome (MODS) according to dynamic changes in individual biomarker levels (a) syndecan-1 and (b) thrombomodulin between prehospital and in-hospital time points.
Significance is indicated according χ^2 test for trend.